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At the edge of darkness and light: macrozoobenthic assemblages of streams in the entrance zone of caves in the Western Caucasus

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Abstract

A study was conducted on the macrozoobenthos fauna of 15 watercourses flowing through the caves of the Duripshski, Gumistinsko-Panavskiy and Dzhalski speleo districts in Abkhazia (Western Caucasus). Assemblages inhabiting the inner part of caves, entrances and surface biotopes are described. A total of 84 species of aquatic invertebrates were identified in the surveyed streams, with 23 species belonging to the ecological group of stygobionts, 17 classified as stygophiles, and 44 stygoxenes. The species richness and abundance of stygoxenes exhibited a decline from surface stations to stations situated deeper within the caves. Stygobionts were found to be most diverse and abundant within subterranean cavities. The number of stygophile species was found to be highest in the ecotone zone. The similarity of the species structure of macrozoobenthos assemblages was the highest in watercourses flowing from neighboring caves and was mainly determined by the distance factor between sites. At the taxonomic level of families, on the contrary, a large proportion of structural variation in assemblages was attributed to the light factor. Ecotone assemblages, although having their own specificity, occupied a dependent position in relation to surface and subterranean. It is substantiated that illuminance is the primary regulatory factor in the inhibition of the penetration of surface and subterranean faunas into habitats that are not typical for them. The active and passive ways of the invasion of stygobionts into the surface biotopes and of stygoxenes into the subterranean cavities are discussed.

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Keywords
Ecotone, ecological factors, spatial distribution, stygobionts, stygophiles, stygoxenes

Introduction

Subterranean ecosystems generally are considered to be ecologically stable and are characterized by low energy levels. However, research in this field has revealed a greater diversity of organizational types, each distinguished by the absence of light (Malard et al. 2023). The cave entrance zone, which marks the boundary between the surface and the underworld, occupies a distinctive position among these ecosystems. The characteristics of cave entrances as transitional areas are consistent with their characterization as ecotones (Gibert 1997; Prous et al. 2004; Culver 2005). The concept of ecotones was developed by E.P. Odum (1953) in the 20th century. The concept implies the existence of interactions between two or more habitats. These interactions are between different continua which accommodate biological communities that utilize different energy flows and are subject to different environmental influences (Howarth 1993). These interactions can define unique habitat features that do not exist in any of the contiguous environments (Kark and van Rensburg 2006; Kark 2017). The concept of ecotones has been applied not only to cave entrance areas, but also in the study of the fauna of springs (Moseley 2009; Culver and Pipan 2014; Manenti and Piazza 2021; Manenti et al. 2023).

Located in semi-darkness, the entrance ecotone combines the environmental conditions of surface and subterranean habitats. Surface habitats are distinguished by a rich abundance of plant organic matter and dynamic environmental conditions, while subterranean habitats are characterized by a scarcity of food resources and more stable environmental conditions (Prous et al. 2004, 2015). The cave entrance zone is subject to both daily and seasonal changes in air temperature, humidity, and light, although this variation is less pronounced than those observed at the surface. Consequently, the entrance zone exhibits greater variability than deeper cave regions, yet it remains more stable than the external environment. The presence of light, even if it is reflected, enables the existence of photosynthetic organisms and even higher plants in the entrance zone (Serena and Meluzzi 1997; Pentecost and Zhaohui 2001; Culver and Pipan 2009). It has been demonstrated that illuminance plays an important role for groundwater inhabitants as a marker of the hazards they may encounter once on the surface. Furthermore, there are studies that consider the cave entrance area as an ecotone in the context of surface fauna studies (Prous et al. 2004, 2015; Pellegrini and Ferreira 2016) and freshwater fauna of cavern-type cenotes (Angyal et al. 2022). A number of studies examining the change in groundwater flow fauna along a gradient of conditions (Chertoprud ES et al. 2016; Culver and Pipan 2019; Borisov et al. 2021) have noted that the extent of the entrance watercourse ecotone is largely determined by the geomorphology of the cave, as well as the direction and velocity of watercourse flow (Borisov et al. 2021).

The majority of studies addressing the interaction between subterranean and surface fauna have focused on individual caves with distinctive characteristics, which makes it difficult to generalize. A number of fundamental questions remain unresolved. Does the ecotone zone exhibit a mixture of subterranean and surface faunal elements, or is another specific species complex dominant? How do the integral characteristics (species richness, abundance, dominance structure) of the aquatic community formed in the ecotone zone differ from the neighbouring ones? Furthermore, it is crucial to ascertain whether stable trophic relationships are formed in the ecotone zone that differ from those in the depth of the cave and on the surface? The study of aquatic communities in the contact zone of subterranean and surface faunas, carried out concurrently across a series of caves, could provide answers to these questions.

This study was carried out in the Western Caucasus, a region characterized by an abundance of karst landforms, including large karst caves, some of which have been identified as the deepest caves on the planet (Dublyansky et al. 1987; Klimchouk 2018; Asanidze et al. 2019). The Western Caucasus region is home to a diverse array of aquatic invertebrates, with more than 110 species having been documented, of which about 90% of which are endemic to the region (Kniss 2001; Shumeyev 2008; Sidorov 2014; Vinarski et al. 2014; Barjadze et al. 2015; Sidorov et al. 2015a, b; Turbanov et al. 2016; Marin 2018; Chertoprud EM et al. 2020; Grego et al. 2020; Marin and Turbanov 2021 etc.). Despite an increasing interest in the region's fauna, the ecological features of the functioning of cave ecosystems in this region are still poorly studied.

This research was devoted to analysing the structure and spatial distribution of macrozoobenthos assemblages in the watercourses from caves of Abkhazia (Western Caucasus). Here, we tested the hypothesis that the macroinvertebrate assemblages in the cave entrance ecotone may significantly differ from the assemblages in the inside cave part and in the surface, both in terms of ecological groups and species compositions as well as number and dominance of organisms. The objective of the present study was to investigate the macrozoobenthos assemblages of the cave ecotones, to establish the nature of mixing between subterranean and surface faunas, and to identify the environmental factors regulating it. The definition of key environmental factors was carried for species, genus and family structures of assemblages separately, in order to assess which factors are significant for each taxonomical level and detecting role of local endemism.

Material and methods

Explored area

The present study focused on the Duripshski, Gumistinsko-Panavskiy and Dzhalski speleo districts (Tintilozov 1976) of Abkhazia, where a total of 15 subterranean watercourses flowing from the caves were investigated (Table 1, geographical coordinates of caves see Suppl. material 1: table S1). The locations of the examined caves are shown

on Fig. 1. Eight of these caves were found to be composed of conglomerate rocks, while six were classified as carbonate caves, with a minor admixture of clastic rocks (Gergedava 1990). Conglomerate rocks are consisting of gravelly debris cemented by limestone or marl. Such clastic rocks are most susceptible to mechanical failure and erosion (Schön 2004). The erosive action of ground and surface waters leads to the formation of both individual cavities and entire conglomerate speleo massifs, which are distinguished by a high prevalence of karst funnels characterized by collapsed type entrances (Gerghedava 1986). The caves of the Duripshi (Gvozdetsky 1954) and Dzhali plateau (Mgeladze 1966) are among those studied in the present study. Carbonate rocks free from clastic inclusions are more susceptible to chemical denudation (Tintilozov 1976; Dublyansky et al. 1990). The Adzaba, Tsebeldis satavis, Shua Shakurani, Kveda Shakurani, Abrskili and Otapistavi caves are examples of such formations.

The present study explored five caves in the territory of the Duripshsky Speleo District (Table 1, Fig. 1). All caves were located on the territory of the Duripshi karst plateau. The total length of the Adzhimchigrinskaya Cave passages that were the subject of this study exceeds 1 km, whereas the length of the passages in the other caves does not exceed 300 m (Table 1). In Kveda Likhni Cave, Tvanba-I Cave, and Adzhimchigrinskaya Cave, streams can be found under the scree in the entrance cave zone, reappearing on the surface from under the rubble outside the cave. In the case of Zeda Likhni Cave, the watercourse flowed unimpeded through the entrance cave zone (Fig. 2C), thus making it accessible for exploration.

A total of nine caves have been the subject of study in the Gumistinsko-Panavski Speleo District (Table 1, Fig. 1). Adzaba Cave, while comparatively modest in length (Table 1), features an expansive entrance cave zone (Fig. 2A). Tsebeldis satavis Cave, Shua Shakurani Cave and Kveda Shakurani Cave caves are horizontal and possess a flooded central gallery with a stream flowing along the entire passage. The largest of these caves, Kveda Shakurani Cave, is characterized by an extensive and elongated entrance cave zone (Fig. 2F), with the total length of the explored passages exceeding 1.3 km. Abrskili Cave and Otapistavi Cave are represented by horizontal branching gallery systems, with the largest of these supporting a river. The total length of the explored part of Abrskili Cave was nearly 2.5 km, while the length of Otapistavi Cave's passages is about 500 m. Both caves have well-defined entrance cave zones through which the river flows out.

Three caves have been the focus of detailed study in the territory of Dzhalsky Speleo District (Table 1, Fig. 1). These caves are characterized by their small length (Table 1), horizontal orientation and proximity to the surface. Thina Cave (Fig. 2B) and Gupskaya Cave have a medium-sized entrance cave zone (Table 1), and the stream flowing out of them continues to flow further on the surface. In Dzhalskaya-I Cave, the connection with the surface is facilitated by a fissure in the vault above the stream (Fig. 2E), through which light, sediment, plant detritus and surface fauna can enter. An exception to this is represented by an old old railway tunnel, not functioning for more than 10 years, which was investigated in the vicinity of the abandoned mining village of Akarmara (Fig. 2D). The tunnel is distinguished by the presence of groundwater seepage along its walls, leading to the formation of a permanent stream that flows

Table 1. Main characteristics of the studied caves, speleo district zoning according to Tintilozov, 1976. (*–according to Gergedava (1990)). Symbols: ×–access to the watercourse in the entrance zone was absent. Abbreviations: L–length of the cave, H and W–height and weight of the entrance.

№	Name	L*	H / W*	Altitude a. s. l.	Rock types / anthropogenic impact
Duripshsky Speleo District					
1	Zeda Likhni Cave	310	3 / 2	213	conglomerate / absent
2	Kveda Likhni Cave	260	×	35	conglomerate / absent
3	Tvanba-I Cave	80	×	245	conglomerate / absent
4	Adzhimchigrinskaya Cave	1025	×	50	conglomerate / absent
5	Tarkili Cave	235	2 / 3	245	conglomerate / absent
Gumistinsko-Panavski Speleo District					
6	Adzaba Cave	145	10 / 5	280	limestone / rarely visited by tourists
7	Tsebeldis satavis (Istočnik Tcebel'da) Cave	300	2 / 11	450	limestone / rarely visited by locals
8	Shua Shakurani Cave	90	4 / 2	270	limestone / absent
9	Kveda Shakurani Cave	1300	13 / 10	230	limestone / rarely visited by tourists
10	Otapistavi Cave	500	3 / 7	240	limestone / visited by tourists
11	Abrskili Cave	2500	6 / 7	210	limestone / visited by tourists and have artificial light
Dzhalsky Speleo District					
12	Thina Cave	no data	2 / 7	160	conglomerate / absent
13	Dzhalskaya-I Cave	210	1 / 2	250	conglomerate / absent
14	Gupskaya Cave	35	2.5 / 1.5	175	conglomerate / absent
Artificial underground constructions					
15	Old railway tunnel	370	5 / 4	350	no data / visited by locals

Figure 1. Map with location of the studied speleo districts and caves (red flowers) of Abkhazia (Western Caucasus). Speleo districts: Gumistinsko-Panavski (brown area), Duripshsky (dark blue area), Dzhal (orange area). Caves: Zeda Likhni Cave (**1**), Kveda Likhni Cave (**2**), Tvanba-I Cave (**3**), Adzhimchigrinskaya Cave (**4**), Tarkili Cave (**5**), Adzaba (**6**), Tsebeldis satavis Cave (**7**), Shua Shakurani Cave (**8**), Kveda Shakurani Cave (**9**), Otapistavi Cave (**10**), Abrskili Cave (**11**), Thina Cave (**12**), Dzhalskaya-I Cave (**13**), Gupskaya Cave (**14**), Old railway tunnel (**15**). Map prepared in QGIS software using the resource https://srtm.csi.cgiar.org.

Figure 2. The entrance cave zones: **A** Adzaba **B** Thina Cave **C** Zeda Likhni Cave **D** Old railway tunnel **E** Dzhalskaya-I Cave **F** Kveda Shakurani Cave.

a distance of 60–70 metres through the tunnel before exiting. Concomitant with the groundwater, the subterranean dwellers enter the stream.

The Abrskil and Otapistavi caves are the most frequently visited by tourists. The artificial lighting in the Abrskil Cave extends for a distance of 400 meters from the entrance, which is less than a quarter of the total length of the cave. In the Otapistavi Cave, which is also often visited by tourists, human impact is limited to the construction of wooden walkways with a total length of about 50 meters, located almost 100 meters past the cave entrance. At the time of our study, the cave had no artificial lighting. The Kveda Shakurani Cave and Adzaba Cave are visited periodically by tourists but have no artificial structures inside. The Tsebeldis satavis Cave serves as a water source for the nearest village. Other caves were visited only occasionally by local residents or unorganized tourists, primarily in the entrance zone of the cave.

Sampling strategy

The material was collected during February 2020 (caves: № 1-5, Table 1), February 2018 (caves № 6-11, Table 1) and March 2021 (caves № 12-15, Table 1) – this is all early Spring for the Caucasus region. The studied cave streams were autochthonous, in which the water is derived from the endokarst area (Gillieson 1996). In some caves was also insignificant surface water flowing through cracks in the ceiling and walls. Where accessible, samples were collected at three stations, which were located relative to the entrance cave zone as follows:

(1) Inner (subterranean) stations, were located upstream of the cave entrance. In all cases, these stations were located inside the cave and outside the photic zone (complete darkness). The immediate distance of the collection point from the cave entrance depended on the shape and size of the entrance and the nature of the watercourse. A total of 15 quantitative samples were collected from each of the caves.

(2) Entrance stations were located in the ecotone zone, most often several metres directly inside from the cave entrance. These stations were in partial sunlight. A total of 12 quantitative samples were collected 12 caves. These stations were absent in Kveda Likhni Cave, Tvanba-I Cave, and Adzhimchigrinskaya Cave (Table 2). In these caves, the watercourse in the entrance cave zone was obscured by rubble and thus not accessible for investigation.

(3) Surface stations were located downstream of the cave entrance. These stations were located in the photic zone outside the cave. A total of 11 quantitative samples were collected, with one sample collected from each cave. In four cases (Table 2), samples were not collected, either due to the hidden flow of watercourse or due to limitations in research time.

Table 2. Number and species richness (ind./m^{2*} / number of species) of macrozoobenthos in studied caves. Symbols: – sampling station absent in this zone.

№	Name	Stations		
		External	Entrance	Inner
1	Zeda Likhni Cave	264 /16	28 / 4	52 / 1
2	Kveda Likhni Cave	1842 / 5	–	42 / 5
3	Tvanba-I Cave	286 / 5	–	36 / 7
4	Adzhimchigrinskaya Cave	1238 / 10	–	34 / 5
5	Tarkili Cave	–	132 / 13	40 / 7
6	Adzaba	320 / 10	82 / 14	176 / 7
7	Tsebeldis satavis Cave	–	158 / 12	18 / 2
8	Shua Shakurani Cave	–	140 / 15	122 / 14
9	Kveda Shakurani Cave	206 / 10	82 / 13	108 / 7
10	Otapistavi Cave	396 / 13	48 / 6	42 / 5
11	Abrskili Cave	536 / 15	64 / 4	42 / 4
12	Thina Cave	220 / 13	82 / 12	8 / 3
13	Dzhalskaya-I Cave	–	84 / 6	80 / 5
14	Gupskaya Cave	48 / 8	66 / 10	114 / 2
15	Old railway tunnel	34 / 6	60 / 5	128/ 5

* Number of individuals collected per station–ind./m^2 / 2.

The high heterogeneity of the biotopes and low values of faunal abundance and species richness often make it difficult to carry out ecological studies in caves to a full extent. In order to obtain a comprehensive understanding of the structure of the assemblage at each station, mixed quantitative samples were taken (one mixed sample per station). Each mixed sample included organisms from three sites, located at intervals of 1–3 metres from each other. At each station, the samples covered both the areas with the maximum depths and those at the water edge. The predominant substrate types were stones, clayed sand, and calcified rimstone walls. All aquatic invertebrates from the sampling area were collected with manual hemisphere sampler–sieve on a rigid frame (diameter 11 cm and mesh size 0.5 mm). The total area of one mixed quantitative sample was 0.5 m^2. All aquatic organisms were preserved in 90% ethanol. The sampling protocol followed the classic scheme used to study freshwater invertebrates (for example, Chertoprud et al. 2016; Walseng et al. 2018; Borisov et al. 2021).

At each station, the main hydrological characteristics of the water inflow, type of sediments, altitude a.s.l. and illuminance (numerical score) were measured. Additionally, in all caves water temperature, total mineralization (ppm) and pH were determined (See Suppl. material 1: table S1). The last three parameters were measured in eight reference caves at each station, and in the remainder of the caves measurements were performed in one or two stations only. A Hanna portable water analyzer (HI 98129) (Germany) was used to measure environmental parameters. The altitudes of cave entrances were estimated using a Garmin eTrex 30 GPS navigator (Lenexa, Kansas State, United States).

Ecological groups

There are currently about a dozen nomenclatures of subterranean inhabitants, which are mainly based based on the Schiner–Racovitza system (Sket 2008; Trajano and Carvalho 2017). Based on published data (Kniss 2001; Shumeyev 2008; Sidorov 2014; Vinarski et al. 2014; Bardjadze et al. 2015; Sidorov et al. 2015a, b; Turbanov et al. 2016; Chertoprud ES et al. 2020; Chertoprud EM et al. 2023), species were divided into three ecological groups, for which the terms "stygobionts", "stygophiles", and "stygoxenes" were used. The available data don't allow to use more detailed classifications (such as the one proposed by Sket 2008), which includes two groups of troglophiles. In dividing species into groups, were used the classical approach and nomenclature of Schiner–Racovitza system, focusing on the ecological features of species.

Stygobionts form populations only in subterranean habitats, outside of which they occur either accidentally or for a short time. In addition, they show adaptations to the subterranean lifestyle: depigmentation, absence of eyes, etc. The specific morphological adaptations of stygobionts limit their colonization of surface assemblages, making them vulnerable to sighted predators and the negative effects of ultraviolet radiation (Fišer et al. 2014). Although troglomorphisms are frequently present in exclusively subterranean species, these two phenomena (troglomorphisms and being a troglobiont) may be the result of independent biological phenomena (Trajano and Carvalho 2017).

Stygophiles, in turn, differ from stygoxenes in their ecological adaptations to life in subterranean cavities, such as the ability to survive and complete a full life cycle in oligotrophic cave environments. Stygoxenes are surface organisms that have accidentally entered caves. In order to avoid confusion, stegoxenes were referred to as all surface organisms in this research.

Taxonomy

The species composition and abundance were determined in each sample. All collected organisms were identified to species level. Reference material representing most of the invertebrate groups and kept at the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) and the Zoological Museum of the Lomonosov Moscow State University was used for species identifications of stygobionts. All hydrobionts were studied using a Carton SPZ-50 microscope (Carton Optical Industries, Ltd, Kanagawa, Japan), and photographs were taken using a Toupcam 9.0 MP digital camera (Hangzhou ToupTek Photonics Co., Ltd, Hangzhou, China). If necessary, specimens were completely dissected on glass slides filled with glycerol and then mounted on Faure–Berlese's mounting medium (for details see Krantz 1978) and studied using an Olympus CX21 microscope.

Identification keys are only known for the Caucasian members of the genus *Niphargus* (Birstein 1952), the family Typhlogammaridae (Starobogatov 1995; Sidorov 2018), as well as stygobiotic hydrobiidae (Starobogatov 1962; Grego et al. 2020). However, due to the high probability of new species encounters, in some cases no exact identification was possible based on these publications. Therefore, stygobiont identification was mainly based on regional taxonomic articles (Birstein 1952; Sidorov 2014; Vinarski et al. 2014; Sidorov et al. 2015 a, b; Vinarski and Palatov 2019; Marin and Turbanov 2021; Chertoprud et al. 2025 etc.). The determination of the aquatic fauna in the surface sections of the watercourses was carried out on the basis of taxonomic keys presented in monographs: Porfirieva and Dyganova (1987), Kluge (1997), Ivanov et al. (2011), Lantsov (1999) Makarchenko and Makarchenko (1999) Teslenko and Zhiltzova (2009), Timm (2009), as well as a number of articles on the systematics of individual groups: Karaman and Pinkster (1977), Berthélemy (1979), Godunko et al. (2015).

Statistical analysis

The similarity of assemblages was assessed at three taxonomic levels: species, genus and family. Pairwise similarity of the taxonomic composition from different samples was evaluated using the Bray-Curtis index for quantitative data (Magurran 2004). To evaluate the effects of environmental factors on the assemblages structure, we used DistLM (distance-based linear models) test. Distance-based redundancy analysis (dbRDA) plot was used to visually depict the results of DistLM. Our environmental data set contained six variables: location (estimated as the distance of the cave entrances from each other in metres), illuminance, altitude a.s.l., pH, temperature and total mineraliza-

tion. These factors were included to DistLM test. As it was not technically possible to measure luminosity intensity for all sampling points for all the caves during the fieldwork, factor of illuminance was represented as three binary variables: darkness, twilight and light. It generally coincides with three different zones of stations: surface, ecotone and subterranean. The binary variables took a value of "1" for samples in which this category occurred and zero ("0") otherwise. First, marginal tests were performed to determine the effect of each variable on the variation in species assemblage structure. Then, the best-fitting model was selected using the adjusted R^2. This criterion can include in the model all the predictor variables considering in the data analysis so that it ignores the concept of parsimony. Thus adjusted R^2 is more useful criterion for model selection since it takes into account number of variables in the model (Anderson et al. 2008). Sequential tests were provided for each variable that was added to the model. A dbRDA (distance-based redundancy analysis) analysis was used to ordinate the fitted values from a given model. The analysis was performed in Primer and Permanova+ PRIMER-E, Plymouth, UK (Clarke and Gorley 2006).

The validity of the selected sample groups on a gradient of key environmental factors was confirmed by the ANOSIM test. The coordinates of species / families in the redundancy analysis (dbRDA) space were calculated using the weighted averaging method based on the primary data matrix and the variables (stations) coordinates exported from PRIMER. The obtained coordinates of species and families were visualised on scatter plots in the space of the dbRDA1 and dbRDA2 axes. The SIMPER procedure was used to identify the families of species that contributed most to the pattern of differences between macrozoobenthos assemblages from different illuminated areas (subterranean–dark, entrance–twilight and surface–light).

The DistLM test, dbRDA analysis and ANOSIM test were performed in PRIMER 7 analytical software (Primer and Permanova+ PRIMER-E, Version 1.1.0, Plymouth, UK) (Clarke and Gorley 2006). For the SIMPER procedure, the software program PAST version 4.02 (Hammer et al. 2001) was used.

Results

Species richness and abundance.

A total of 84 species of aquatic invertebrates were found in the stream at the explored stations: class Turbellaria–2; subclass Oligochaeta–4; subclass Hirudinea–4; class Gastropoda–13; class Bivalvia–2; subphylum Crustacea orders: Isopoda–1; Amphipoda–12; Decapoda–3; class Insecta orders: Odonata–1; Ephemeroptera–5; Plecoptera–4; Coleoptera–8; Trichoptera–13; Diptera–13 species (See Suppl. material 1: table S2). Among them, 23 species were classified as stygobionts, 17 as stygophiles and 44 as stygoxenes based on available literature data. Of the 61 species of stygophiles and stygoxenes, the majority (44 species) were comprised of larvae and imago of insects (See Suppl. material 1: table S2). Some typical species from three ecological groups are presented in the Fig. 3.

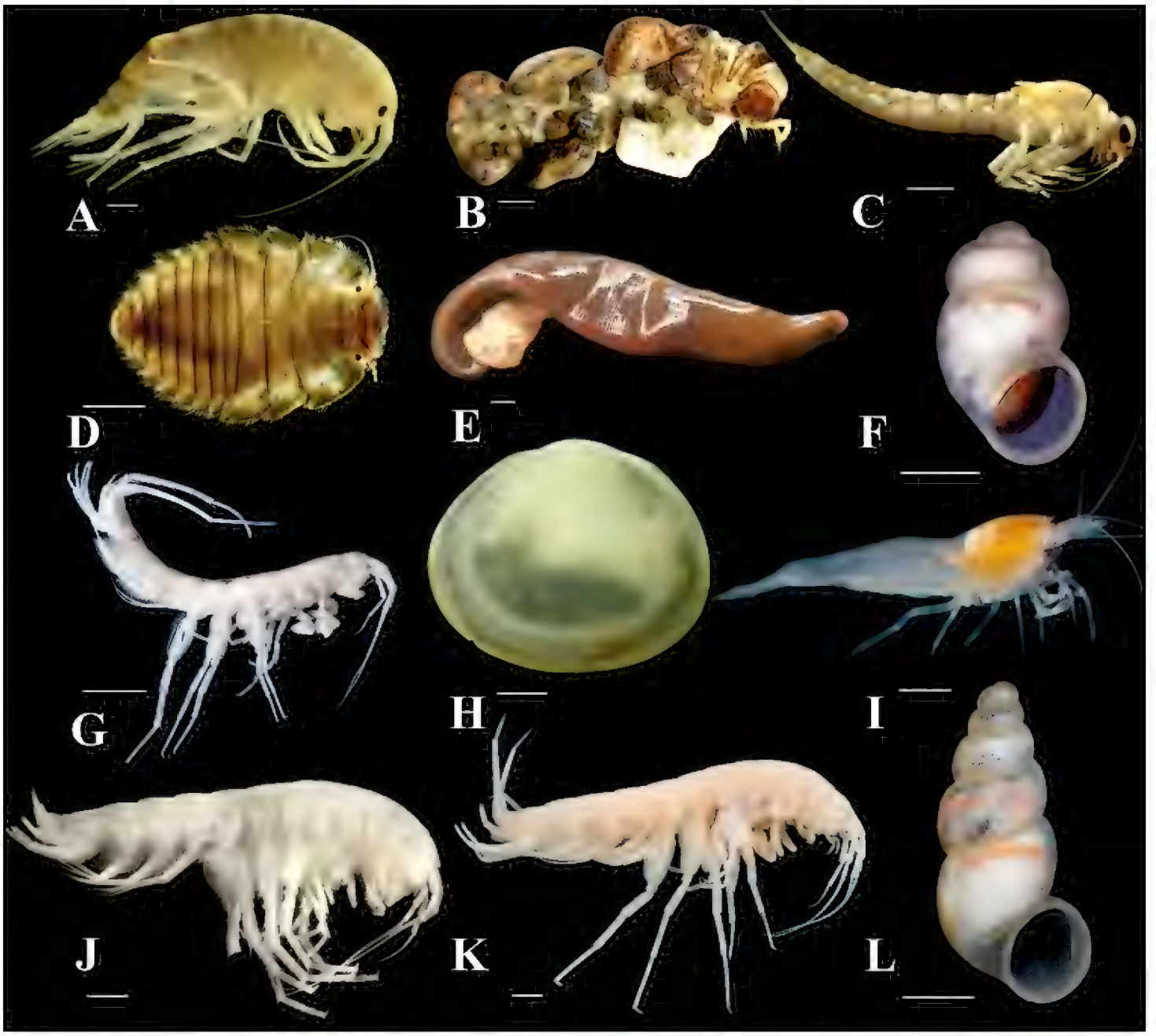

Figure 3. Species from the caves of Abkhazia (Western Caucasus): **A–C** stygoxenes **D–F** stygophiles **G–L** stygobionts: **A** *Gammarus caucasicus* (Adzhimchigrinskaya Cave) **B** *Lithax incanus* (Tarkili Cave) **C** *Baetis* cf. *gemellus* (Kveda Shakurani Cave) **D** *Odeles* sp. (Shua Shakurani Cave) **E** *Trocheta* sp.4 (Adzaba Cave) **F** *Tschernomorica lindholmi* (Gupskaya Cave) **G** *Niphargus* cf. *latimanus* (Kveda Likhni Cave) **H** *Euglesa ljovuschkini* (Tsebeldis satavis Cave) **I** *Xiphocaridinella osterloffi* (Kveda Shakurani Cave) **J** *Zenkevitchia yakovi* (Kveda Shakurani Cave) **K** *Niphargus* cf. *magnus* (Gupskaya Cave) **L** *Caucasopsis* sp. (Thina Cave). Scale bars: 1 mm (**A–E; G, I–K**); 0.5 mm (**F, H, L**).

Outside the caves, in the external zone of the watercourse, 58 species were found; in the entrance area 51 species were found; inside the caves live 45 species (Fig. 4A). The number of stygoxene species decreased from external stations to stations deep inside caves. Conversely, the opposite trend was observed for the species richness of stygobionts (Fig. 4A). The greatest diversity of stygophiles was observed at stations located within the ecotone zone; however, stygoxenes predominated in terms of species number (Fig. 4A). Furthermore, trends in species richness variability depending on the degree of watercourse illuminance were also noted for individual macro taxa. Thus, the number of insect species decreased from surface stations to stations inside the cave, while the number of crustacean species exhibited an opposite trend. (Fig. 4B).

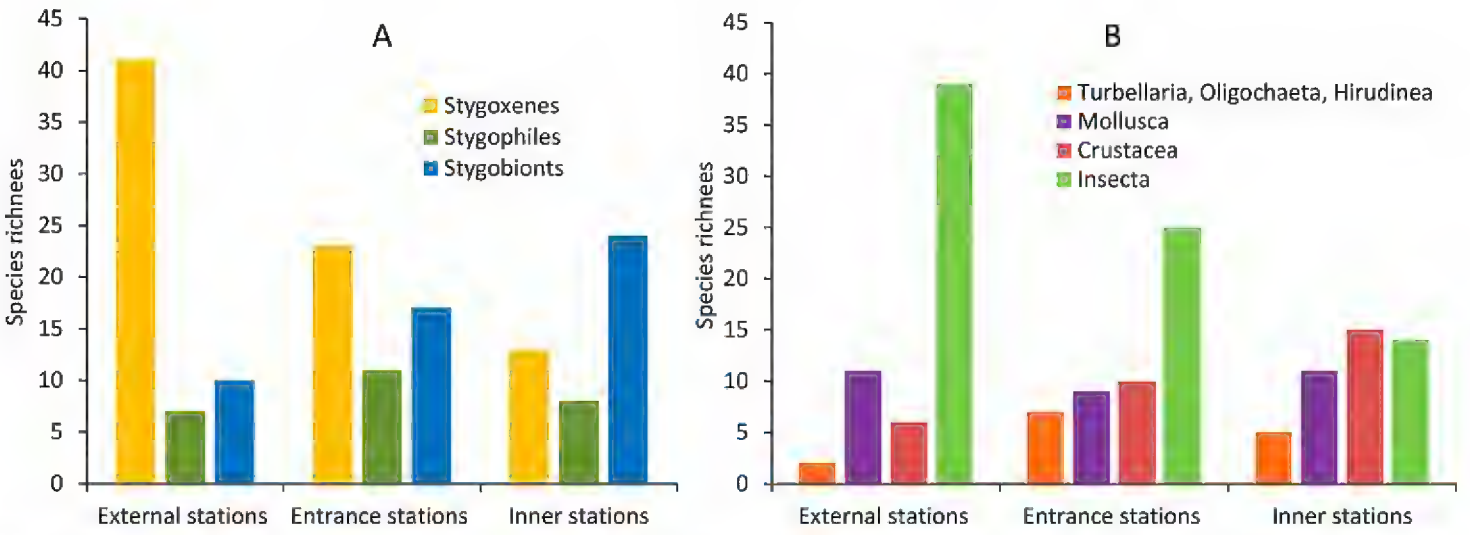

Figure 4. The total species richness of various ecological (**A**) and taxonomic groups of organisms (**B**) in zones of differing illuminance according on position relative from the cave entrance.

The highest number of organisms (1842 ind./m^2) were recorded at external stations outside the caves. The average hydrobiont abundance at the outer stations (480 ± 542 ind./m^2) generally exceeded that observed in the ecotone zone (83 ± 37 ind./m^2) and at the inner stations (68 ± 49 ind./m^2). The predominant contribution to the quantitative macrozoobenthos assemblages in the area outside the caves was that of stygoxenes (Fig. 5A), of which the dominant species were amphipods and insects (Fig. 5B). In the ecotone zone, the contributions to the total abundance of stygoxenes, stygobionts and stygophiles were found to be similar (Fig. 5A). The majority of species observed at stations within the cave were stygobionts, with stygophiles and stygoxenes represented by single finds (Fig. 5A). The contribution to total crustacean abundance was higher in areas outside the caves, while mollusks were more prevalent in the subterranean cavities (Fig. 5B). The proportion of insects in total assemblages was found to be constant both outside the cave and in the ecotone zone. However, the mean insect abundance was consistently higher outside the cave than in the entrance zone–92 ± 102 and 27 ± 25 ind./m^2, respectively.

The results pertaining to the abundance and species richness of the invertebrate macrofauna in each of the caves examined are set out in Table 2, with the division by the three types of station illumination given.

Environmental factors regulating assemblages

The influence of environmental factors on the structure of assemblages at three taxonomic levels (species, genera and families) was assessed by DistLM analysis (See Suppl. material 1: table S3). The analysis revealed that all environmental factors together accounted for 32–35% of the variation in assemblage structure (See Suppl. material 1: table S3). At all taxonomic levels, the most significant environmental factors were the cave location, illuminance and pH ($p < 0.05$). The influence of illuminance gradually increases with increasing taxonomic level from species to families, while the role of cave location decreases in this series (See Suppl. material 1: table S3). Consequently,

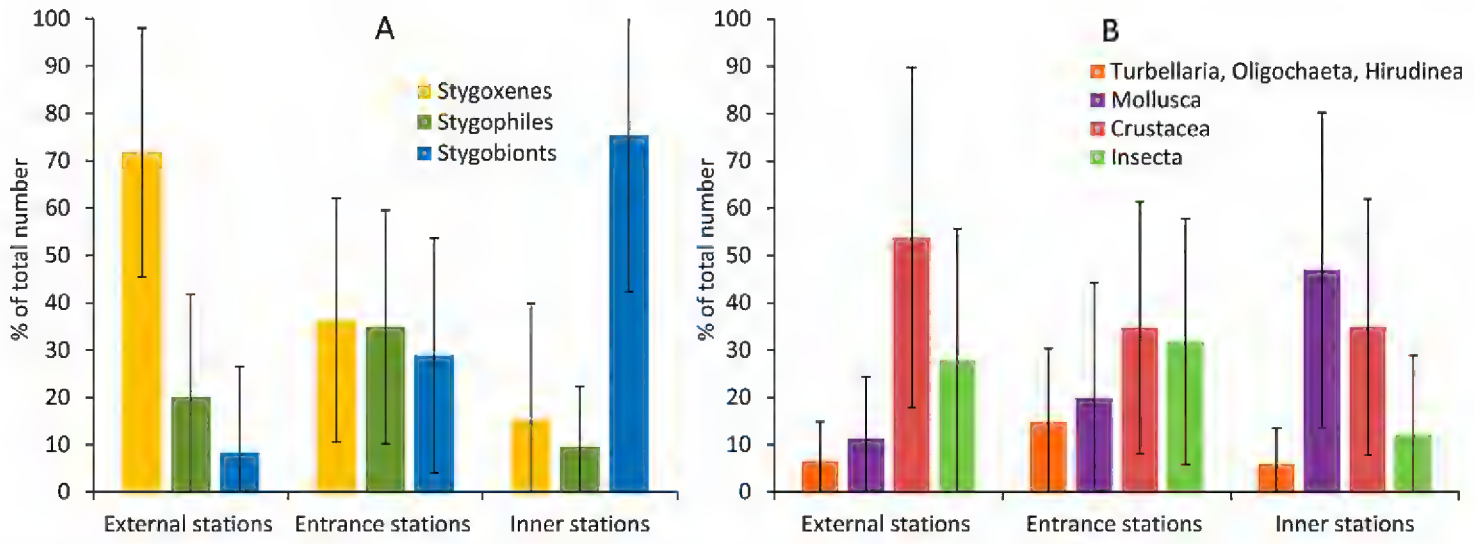

Figure 5. The parts of number of various ecological (**A**) and taxonomic groups of organisms (**B**) in zones of differing illuminance according on position relative from the cave entrance.

illuminance explains only 7.9% of variability in species structure, 11.9% in genera structure and 16.2% in family structure of assemblages. Conversely, the cave location factor accounts for 15.1% of the variation in species structure, 6.5% in genera structure, and only 4.4% in family structure. pH contributes approximately 6% to the variability of assemblages, with a slightly higher contribution of 7.9% observed for genera and family structure. It is important to note that a significant proportion of assemblage variations remain unexplained, which is due to the high heterogeneity of the other environmental conditions in the biotopes studied.

The dbRDA diagrams (Fig. 6A, B) demonstrate a divergent grouping of assemblages evaluated at the two most contrasting taxonomic levels, species and families. The species structure of assemblages is more significantly influenced by the factor of distance between the studied caves, which corresponds to the longest vector on the diagram (Fig. 6A). In this regard, the points corresponding to stations from one cave are located in relatively close proximity to each other. The most densely concentrated group is observed in the upper left corner of the diagram, within the south-eastern part of the Gumistinsko-Panavsky and Dzhalsky Speleo Districts (caves № 10–15). The structural organization on family level is more analogous in stations with analogous illuminance levels. The points corresponding to assemblages from subterranean biotopes are concentrated in the lower left quadrant of the diagram, while those from surface habitats form a cloud in the upper right quadrant (Fig. 6B). Assemblages from the ecotone zone occupy an intermediate position, slightly mixed with the cloud of surface habitat points.

Environmental preference of species and families

The similarity of species distribution is related with the factor of distance between the caves (Fig. 6C). Thus, stygobiont species inhabiting closely located caves of the Gumistinsko-Panavski Speleo District form clusters of points in the lower (fauna of caves 7, 8, 9) and middle left (fauna of caves 10 and 11) part of the diagram. By contrast,

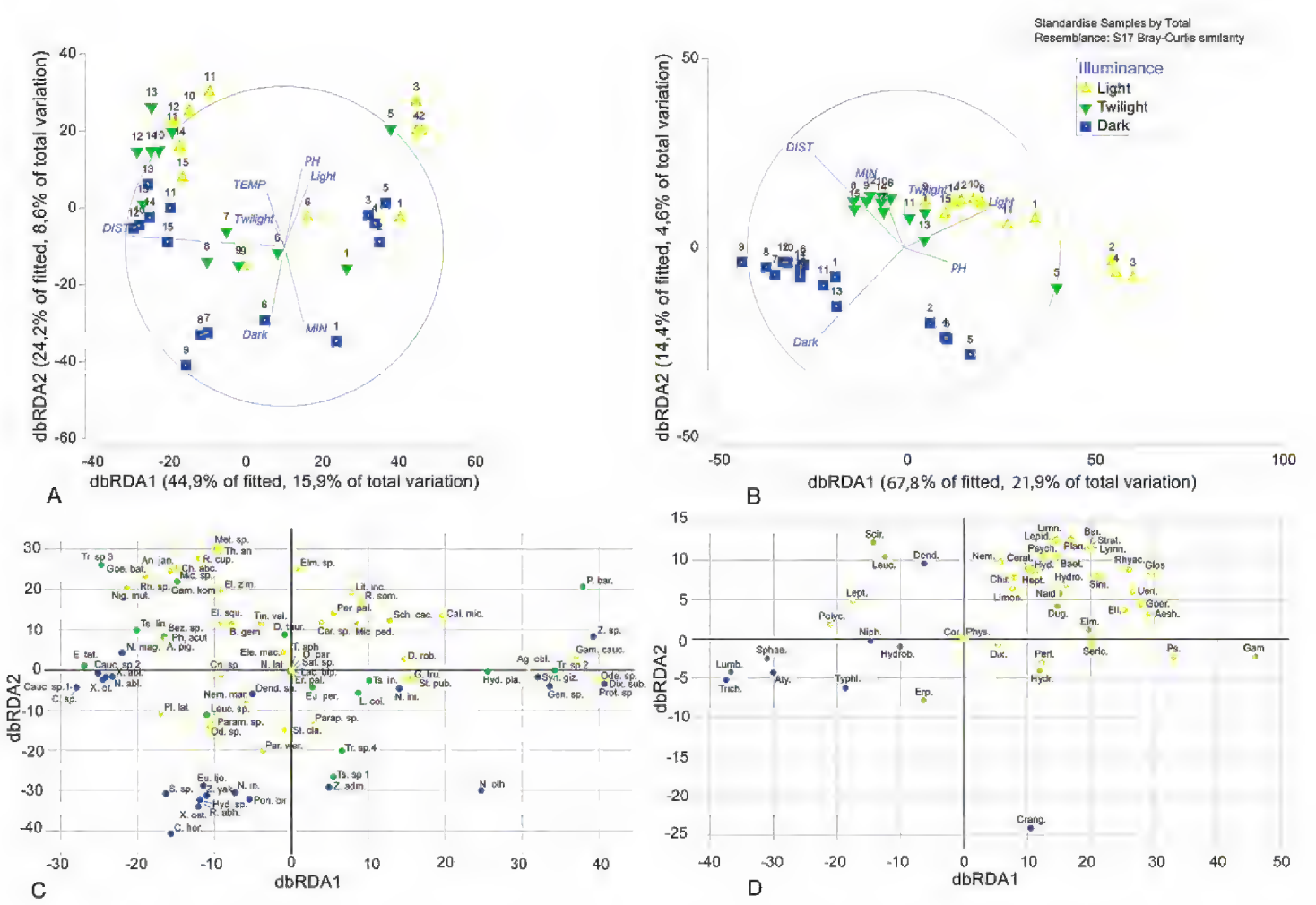

Figure 6. dbRDA ordination of the assemblages structure of caves on the species (**A**) and families (**B**) levels, as well as ordination of species (**C**) and families (**D**) preferences (based on Bray–Curtis similarity index) factored with illuminance ranges: light–fully illuminated (surface), twilight–half-light (ecotone), dark–darkness (subterranean). Abbreviations: DIST–location of cave, TEMP–temperature of water, MIN–total mineralization (ppm), PH–acidity; families names: Dug.–Dugesiidae, Dend.–Dendrocoelidae, Naid.–Naididae, Lumb.–Lumbriculidae, Erp.–Erpobdellidae, Hydrob.–Hydrobiidae, Lymn.–Lymnaeidae, Ell.–Ellobiidae, Phys.–Physidae, Plan.– Planorbidae, Sphae.–Sphaeriidae, Trich.–Trichoniscidae, Gam.–Gammaridae, Niph. –Niphargidae, Crang.–Crangonyctidae, Typhl.–Typhlogammaridae, Aty.–Atyidae, Aesh.–Aeshnidae, Hept.–Heptageniidae, Baet.–Baetidae, Lept.–Leptophlebiidae, Nem.–Nemouridae, Leuc.–Leuctridae, Perl.–Perlodidae, Elm.–Elmidae, Hyd. –Hydrophilidae, Hydr.–Hydraenidae, Scir.–Scirtidae, Psych.–Psychomyiidae, Phil.–Philopotamidae, Hydro.–Hydropsychidae, Polyc.–Polycentropodidae, Glos.–Glossosomatidae, Rhyac.–Rhyacophilidae, Limn.–Limnephilidae, Lepid.–Lepidostomatidae, Seric.–Sericostomatidae, Goer.–Goeridae, Ber.–Beraeidae, Uen.–Uenoidae, Limon.–Limoniidae, Chir.–Chironomidae, Cerat.–Ceratopogonidae, Sim.–Simulidae, Psycho.–Psychodidae, Dix.–Dixidae, Strat.–Stratiomyidae. The abbreviations of species names are presented in Suppl. material 1: table S4. Numbers refer to cave numbers in Table 1.

stygobiont species from caves of the Duripshsky Speleo District form a points cloud in the right central region of the diagram (fauna of caves 2–5). The dbRDA diagram of families distribution (Fig. 6D) reflects the pronounced gradients of assemblages composition in the caves, where stygobiont organisms are replaced gradually with surface organisms. The blue points corresponding to families with prevalence of stygobiont organisms from the subterranean portion of watercourses are grouped within the

left section of the diagram (Fig. 6D). The turbellarians of the family Dendrocoelidae, oligochaetes of the family Lumbriculidae (genus *Stylodrilus*), bivalvians of the family Sphaeriidae (certain species of genus *Euglesa* (Fig. 3H)), gastropods of the family Hydrobiidae (*Pontohoratia* and *Caucasopsis* genera (Fig. 3L)), isopods of the family Trichoniscidae, amphipods of the families Niphargidae (Fig. 3G, K), Crangonyctidae and Typhlogammaridae (Fig. 3J), shrimps of the family Atyidae (Fig. 3I) and several other taxa are presented here. In the right part of the diagram the yellow points corresponding to families of the surface part of watercourses were grouped (Fig. 6D). Macrozoobenthos taxa characteristic of surface assemblages are presented in them: amphipods of the family Gammaridae (some species of the genus *Gammarus*), larvae of insects of Ephemeroptera (families Baetidae (Fig. 3C) and Heptageniidae) and Trichoptera orders (families Glossosomatidae, Hydropsychidae, Psychomyiidae, Rhyacophilidae, Sericostomatidae and others), beetles of the families Elmidae (*Riolus*, *Elmis* genera) and Hydrophilidae and other taxa. Families characteristic of the entrance ecotone occupy the central part of the diagram–the green points. The entrance zone is favoured by turbellarians of the family Dugesiidae, leeches of the family Erpobdellidae (Fig. 3E), molluscs of the family Hydrobiidae (genus *Tschernomorica* (Fig. 3F)), plecopteran larvae of the family Leuctridae, beetles of the families Elmidae (genus *Limnius*), Hydraenidae and Scirtidae (Fig. 3D), as well as some other taxa. The points corresponding to these families separate two relatively dense groups of points for surface ones and characteristic of caves.

The ANOSIM test indicates that the structure of macrozoobenthos assemblages differs in different light zones at the taxonomic level of genera and families (Table 3). However, no significant separation of assemblage groups according to the gradient of illuminance factor on the basis of species structure was observed. The R-statistic value was found to be 0.06 for species structure, 0.132 at the genus level, and 0.218 at the family level (Table 3). It was observed that the level of differences increased in direct proportion to the taxonomic level. The greatest differences were observed between surface (external stations) and ecotone assemblages, as well as assemblages of internal stations (Table 3).

In the context of the macrozoobenthos assemblages of the caves under study, differentiating species have been identified (Table 4). The most significant contributions to the differentiation of species structures were made by *Gammarus caucasicus* Martynov, 1932 (25% of total differences) (Fig. 3A), *Gammarus* cf. *komareki* (Schaferna, 1922) (7.8%), *Tschernomorica lindholmi* (Vinarski & Palatov, 2019) (7.8%) (Fig. 3F) and *Trocheta* sp. (5.7%) (Fig. 3E). First three species constituted the most significant abundance proportion of the assemblages. For macrozoobenthos assemblages of streams pats with different illuminance conditions (light, twilight and darkness) differentiating macroinvertebrates families were distinguished (Table 4). The largest contribution to the distinction of assemblages of different photic zones was made by the families of Gammaridae (32.9% of total differences), Hydrobiidae (16.2%), Baetidae (7.3%) and Niphargidae (5.2%).

Table 3. Results of the ANOSIM tests (one-way global and pairwise) for non-random differences between caves assemblages grouped by illuminance, R-values with p < 5% are in bold. Abbreviations of groups: 1–fully illuminated (surface), 0.5–twilight (ecotone), 0–darkness (subterranean).

Factors		R-statistic	p-value, %
	SPECIES LEVEL		
Illuminance, one-way ANOSIM		0.06	7.4
	Groups 1 and 0.5	0.101	7.1
	Groups 1 and 0	0.086	6.2
	Groups 0.5 and 0	0.03	26
	GENERA LEVEL		
Illuminance, one-way ANOSIM		**0.132**	1.2
	Groups 1 and 0.5	**0.165**	2
	Groups 1 and 0	**0.239**	0.6
	Groups 0.5 and 0	0.028	27
	FAMILY LEVEL		
Illuminance, one-way ANOSIM		**0.218**	0.1
	Groups 1 and 0.5	**0.237**	0.7
	Groups 1 and 0	**0.356**	0.1
	Groups 0.5 and 0	0.042	18.8

Table 4. The most distinctive (> 5% of the explained difference contribution) species of assemblages in different studied caves and families of assemblages from different illuminated areas (SIMPER analysis).

Taxa	Contribution, %	Cumulative, %
	SPECIES LEVEL	
Gammarus caucasicus	25.00	25.00
Gammarus cf. *komareki*	7.81	32.81
Tschernomorica lindholmi	6.69	39.5
Trocheta sp.	5.72	45.22
Overall average dissimilarity		93.24
	FAMILY LEVEL	
Gammaridae	32.92	32.92
Hydrobiidae	16.21	49.13
Baetidae	7.25	56.38
Niphargidae	5.22	61.6
Overall average dissimilarity		85.61

Discussion

What determines the structure of assemblages: geography or abiotic factors?

The fauna of studied caves of Abkhazia has a relatively high diversity of fauna characteristic of the southwestern coastal slopes of the Caucasus Range (Palatov et al. 2016; Palatov and Chertoprud 2018), where a subtropical climate prevails. The macroinvertebrates found belonged to three different ecological groups. These included stygoxenes, which are characteristic of surface habitats (53% of the total species richness), stygobionts inhabiting caves (27%), and stygophiles inhabiting the ecotone zone (20%) (See Suppl. material 1: table S2). Important, that the distribution of organisms at various

taxonomic levels, ranging from species to families, is significantly influenced by different environmental factors. Thus, the geographical factor of cave location emerged as a primary driver of species composition (See Suppl. material 1: table S3). In this regard, most significant similarity in the species composition of assemblages in neighboring watercourses was exhibited (Fig. 6A, C). This finding indicates that dispersal barriers have a substantial impact on the distribution of macrozoobenthos fauna in mountain massifs and speleo districts (Chertoprud ES et al. 2016; Palatov et al. 2016). At the taxonomic level of families, however, the illuminance factor, which accounts for the largest share of structural variations in assemblages (See Suppl. material 1: table S3), was found to be the most significant. Previous research has documented disparities in factor regulation across different taxonomic levels, particularly in the context of tropical marine meiobenthos (Chertoprud ES et al. 2013; Mokievsky et al. 2024). In environments exhibiting high diversity of species, the similarity of the structure of meiofauna assemblages was found to be preserved exclusively at stations located in close proximity to each other. At the same time, the distribution of genera and families was found to be influenced by a complex of abiotic factors related to biotope soil characteristics (Mokievsky et al. 2024).

Macroinvertebrates of cave watercourses in the North Caucasus are characterized by a high level of local endemism (Chertoprud ES et al. 2016, 2020). Even the fauna of cavities of neighbouring river valleys can exhibit substantial differences (or have no common taxa at all) among slowly dispersing subterranean mollusks, amphipods, and shrimps (Chertoprud ES et al. 2016). These island-like habitats are isolated environments, and the "ocean" for their fauna is the land surface, with its inherent environmental features (Culver and Pipan 2008). Therefore, it is not surprising that the degree of similarity in stygobiont composition depends on the geographical proximity of caves to each other. Furthermore, the "founder effect" (De Meester et al. 2002) can lead to the formation of distinct assemblages in streams. This phenomenon occurs when species that are the first to establish in a stream after drying or flooding occupy all available ecological niches, making it challenging for subsequent colonists to penetrate the assemblage (Aarnio and Bonsdorff 1992; Chertoprud ES et al. 2023). Consequently, even in geographically proximate river valleys, the formation of "parallel" communities becomes possible, wherein the same ecological niches are occupied by different, yet functionally analogous, species belonging to the same family (Palatov and Chertoprud 2018). The distribution of hydrobiont families typically exhibits greater breadth in comparison to that of individual species. Within a high-ranking taxon, organisms are united by common morphological and physiological adaptations to living in certain abiotic conditions (Husmann 1966, 1967).

It is evident that the distribution of macrozoobenthos in the studied watercourses also was influenced by other environmental factors besides localization and illuminance. These factors encompassed not only the variables incorporated in the present study (i.e., total salinity, pH, and temperature) (See Suppl. material 1: table S3), but also those that were not–cave geomorphology and lithology (Pacheco et al. 2021), stream hydrology (Pellegrini et al. 2018, 2020), refuges and food resources (Pellegrini

et al. 2018). It is noteworthy that the species composition of hydrobionts has been identified as a reliable indicator of the localization of a watercourse, while the composition of families–of the area of the watercourse in relation to light (surface versus subterranean). This was confirmed by the dbRDA analysis results (Fig. 6).

Are assemblages of ecotone: specific or transitional between surface and subterranean?

Representatives of subterranean cave communities inhabit stable environments with limited food resources, while surface assemblages exist in less stable environments with a more abundant trophic base (Prous et al. 2004). Entrance areas of caves are ecotones that combine features of both bordering environments (Prous et al. 2015). It is reasonable to hypothesize that ecotone assemblages of macroinvertebrates will possess their own specificity, differing considerably from their neighboring cave and surface assemblages. Indeed, it turned out that the ecotone community has its own specificity of species composition and structure of assemblages of hydrobionts.

Stygophiles, which are capable of inhabiting a broad spectrum of light conditions, demonstrate a preference for habitats in twilight (Husmann 1966), and exhibit maximum diversity within the ecotone. The entrance zone is particularly favored by several taxa, including turbellarians of the family Dugesiidae, leeches of the family Erpobdellidae, molluscs of the family Hydrobiidae, plecopteran larvae of the family Leuctridae, and beetles of the families Elmidae, Hydraenidae, and Scirtidae, among others (Fig. 6D). Notable species inhabiting this zone include planaria *Dugesia tauro-caucasica* (Livanov, 1951), leeches of the genus *Trocheta* (Fig. 3E), gastropods of the genus *Tschernomorica* (Fig. 3F), amphipods *Gammarus* cf. *komareki* (Schaferna, 1922), plecopteran larvae *Leuctra* sp., larvae and imago of beetles *Limnius colchicus* Delève, 1963, *Hydraena* gr. *planata* Kiesenwetter, 1849, *Hydraena* sp. and *Odeles* sp. (Fig. 3D). The entrance zone assemblages are dominated by numerous species of stygoxenes, stygobionts and stygophiles. It is in the ecotone that the number of species and contribution to the total abundance of stygoxenes and stygobionts are close (Figs 4, 5). In contrast, surface and subterranean environments are dominated by only one of these ecological groups of organisms. Despite the evident specificity of ecotone assemblages, they still occupy an intermediate position between surface and subterranean species complexes. This is emphasized by the fact that more than half of the total species richness of surface and subterranean fauna is found in the ecotone (62.5% and 60.0%, respectively). However, the composition of the hydrobiota in the entrance zone exhibits a greater similarity to surface habitats, with 76.1% of the ecotone fauna comprising species found in surface habitats. In some cases, these species occupied dominant positions both in the entrance part of the watercourse and downstream (See Suppl. material 1: table S2). At the same time, only 58.7% of the ecotone fauna was observed within the depth of the caves. This phenomenon can be attributed to two primary factors: first, active avoidance of the ecotone zone by stygobionts, and second, frequent penetration of adult stygoxene insects into entrancees for the purpose of oviposition.

The claim that species richness and abundance of organisms tends to peak in ecotone habitats (Prous et al. 2004, 2015; Culver 2005; Konec et al. 2015; Thorp 2015), was not confirmed clearly during the present study. In most streams, the diversity and abundance of organisms outside the cave entrance zone was significantly higher than in the cave entrance zone (Table 2). A comparable rise in macrozoobenthos faunal richness downstream has been documented for spring watercourses originating from groundwater (Carroll and Thorp 2014). However, in caves featuring extensive entrancees (Adzaba and Kveda Shakurani caves) the highest species richness was observed in the ecotone zone. Notably, Gupskaya Cave exemplifies this phenomenon, exhibiting ecotone assemblages that surpass those of neighboring caves in both species richness and organism abundance.

Factors determining the organisms spatial distribution–to meet the light or to the depths of the subterranean habitats?

In the ecotone zone, the mixing of surface and subterranean faunas of aquatic invertebrates is observed, and their further dispersal outside the natural habitat is inhibited. However, stygoxenes also were found at stations in the aphotic zone within caves, and stygobionts, in turn, in surface habitats. Encounters of species in nonnative light conditions were observed regularly, but the abundance of these colonists was very low (See Suppl. material 1: table S2). The occurrence of stygobionts outside caves has been documented on multiple occasions. For instance, the shrimp species *Xiphocaridinella* (Marin and Sokolova 2014) and the gastropod *Radomaniola curta germari* (Frauenfeld, 1863) (Perić et al. 2018) have been recorded after floods in streams of the Black Sea and Mediterranean coasts, respectively. Single encounters of stygoxene insect larvae have also been recorded in many subterranean cavities (Venarsky et al. 2012; Culver and Pipan 2019). What are the underlying factors that motivate organisms to leave their natural habitats? Stygobionts usually are found outside caves as a result of seasonal floods and drift, defined as the migration of organisms along with the current. These phenomena play a significant role in the distribution of macrozoobenthos in both surface and groundwater streams (Brittain and Eikeland 1988; Naman et al. 2016). Additionally, the influx of stygobionts into watercourses occurs via hyporheic microcavities outside caves, a phenomenon that is particularly common in spring communities (Malard et al. 2002; Malard et al. 2009; Manenti and Barzaghi 2021). The presence of aquatic larvae of stygoxenes in the aphotic zone often is attributed to passive transport of organisms from the surface through rock fractures and sinkholes along with stream waters (Venarsky et al. 2012; Perić et al. 2018; Culver and Pipan 2019). In the caves investigated, this phenomenon was observed in Tarkili and Shua Shakurani Caves (See Suppl. material 1: table S2). As stygoxenes generally migrate into caves along the direction of water flow (White et al. 2019), the direction of flow into or out of the cave is of significance. Amphipods have also been observed to migrate against the flow of the watercourse (Williams and Hynes 1976; Williams 1977). However, for surface species of amphipods, it was

noted that if a section of the watercourse flows subterranean, it becomes an obstacle for their migrations (Luštrik et al. 2011).

The avoidance of non-specific light conditions by stygoxenes and stygobion ts is evident; however, the mechanisms ensuring such distribution of organisms are debatable.The main factors preventing hydrobionts from colonizing non-native habitats include a lack of food resources (Culver and Pipan 2014), predator pressure (Luštrik et al. 2011; Galbiati et al. 2023; Manenti et al. 2023), light or its absence (Moran et al. 2015), and UV radiation detrimental to pigmentation-deprived stygobionts (Manenti and Barzaghi 2021). These factors offer compelling reasons for organisms to avoid certain habitats; however, they do not serve as suitable behavioural regulators for governing the distribution of faunas. It is more effective for hydrobionts to avoid biotopes where all these unfavorable environmental conditions will affect them in combination. Although illuminance itself does not pose a direct threat, its changes are correlated with gradients of many biotic and abiotic components of habitats.

The absence of light constitutes the most significant factor in determining the ecology of subterranean ecosystems and the evolution of subterranean inhabitants (Culver and Pipan 2015; Konec et al. 2015; Fišer et al. 2016; Angyal et al. 2022). Negative phototaxis has been observed for a considerable number of cave hydrobiont species (Borowsky 2011; Soares and Niemiller 2013; Fišer et al. 2016; Galbiati et al. 2023). The absence of eyes does not act as a barrier to responses to light, as many organisms possess not only vision but also extraocular and non-visual photoreception (Cronin and Johnsen 2016). Behavioural responses to light have been proposed as a mechanism to prevent the dispersal of stygobiont crustaceans of the genus *Niphargus* into surface biotopes (Fišer et al. 2014, 2016; Galbiati et al. 2023). At the same time, being in constant darkness represents a significant stress factor for stygoxene species (Riesch et al. 2011).

Food resources can also act as a factor limiting the distribution of faunas. In oligotrophic cave ecosystems, stygobiont species primarily consume the microbial community, which contains heterotrophic and chemoautotrophic bacteria (Kováč 2018). In the family Atyidae, the pereopods of shrimps are adapted to the collection of bacterial biofilms due to the specific composition and structure of bristles (Page et al. 2007). Many cave gastropods, amphipods, and isopods are also biofilm consumers (Simon et al. 2003). In contrast, surface species inhabiting watercourses have access to a diverse and abundant prey base comprising both animal and plant components (Merritt 1978). The hypothesis posits that surface species displace subterranean species when food resources are abundant in an ecosystem. Conversely, subterranean species gain a competitive advantage when food availability is limited (Culver and Pipan 2009).

The recreational utilization of caves by humans for tourism purposes has been identified as a contributing factor to the instability of the subterranean environment (Pellegrini and Ferreira 2016). There is evidence that artificial lighting of caves, as well as the introduction of substrates from the surface (e.g., wood), leads to eutrophication of subterranean waterways and promotes the invasion of stygoxenes into cavities (Sousa-Silva et al. 2012; Venarsky et al. 2012, 2018). Specifically, the presence of light attracts adult stygoxene insects to the caves, where they subsequently deposit their eggs along the watercourse upstream (Brown et al. 2011). Indeed, in this research, Abrskill

Cave, which has artificial light, larvae of surface Plecoptera were found on rotting wood >100 m from the entrance (Chertoprud ES et al. 2016). A similar situation was observed in another Caucasian cave equipped for tourism, Kumistavi Cave (Georgia), where imago of stygoxene species of Coleoptera and larvae of Plecoptera were found (Chertoprud ES et al. 2020).

Therefore, the processes of mixing of surface and subterranean aquatic invertebrate faunas are regulated by a wide range of environmental conditions, including both abiotic and biotic factors. Anthropogenic impacts pose a threat to cave communities, introducing additional risks of ecosystem transformation. The combined effect of humans and global climate change affecting organic matter fluxes can lead to irreversible transformation of cave communities (Humphreys 2018).

Conclusion

The results of this study highlighted that the hydrobiont assemblages in the ecotone zone had their own specific taxonomic composition, dominance structure and ratio of different ecological groups of organisms. However, the assemblages of the ecotone occupied an intermediate position between the surface and subterranean communities. Over half of the total species richness of the ecotone fauna consisted of species inhabiting subterranean and surface habitats. In the ecotone, the dispersal of surface and subterranean organisms beyond their natural habitat was inhibited. The main regulator of the distribution of the fauna was illuminance, with changes to which are associated gradients of a series of environmental factors that affect the survival of hydrobionts. This factor is particularly important at the family level, given to the similar morphological and physiological adaptations of organisms within macrotaxa. Therefore, installing artificial lighting in caves can lead to the gradual transformation of subterranean assemblages due to the invasion of stygoxene taxa.

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **table S1.** The main characteristics of the studied stations in caves of Western Caucasus Illuminance expressed in scores: 0 – absent (total darkness); 0.5 – twilight; 1 – full light. **table S2.** Distribution of aquatic invertebrates in the streams of studied caves of Western Caucasus. **table S3.** The results from DistLM test, including marginal and sequential tests. **table S4.** The abbreviations of species names for Fig. 6.

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